1.0 Introduction

Commercial and household cleaning products must be labeled to indicate if they are hazardous to the consumer during handling or use. The U.S. Consumer Product Safety Commission (CPSC) typically regulates these products under the Federal Hazardous Substances Act (15 U.S.C. 1261 and 16 CFR 1500) and the Poison Prevention Packaging Act (16 CFR 1700). However, the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 136-136y, 40 CFR 161) requires that cleaning products with an antimicrobial claim register as antimicrobial pesticides with the U.S. Environmental Protection Agency (EPA) Office of Pesticide Products (OPP). To comply with EPA classification and labeling requirements for eye irritation (EPA 2003), a product manufacturer must provide Draize rabbit eye test data (Draize et al. 1944) (40 CFR 158; 40 CFR 161).

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Authorization Act of 2000 (Public Law 106-545, 42 United States Code 285*l*-3) charged ICCVAM with coordinating the technical evaluation of new, revised, and alternative test methods that have regulatory applicability. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) administers ICCVAM and provides scientific support for ICCVAM activities.

In June 2004, the EPA–OPP contacted NICEATM to request a technical assessment of an *in vitro* testing strategy that would meet their need to evaluate, categorize, and label antimicrobial cleaning products (AMCPs) for eye irritation. The AMCP testing strategy comprises three *in vitro* test methods: the bovine corneal opacity and permeability (BCOP), Cytosensor® Microphysiometer (CM), and EpiOcular™ (EO) test methods. The Alternative Testing Working Group (ATWG), a consortium of seven consumer product companies (Clorox, Colgate-Palmolive, Dial, EcoLabs, JohnsonDiversey, Procter & Gamble, and SC Johnson), developed the AMCP testing strategy, coordinated by the Institute for In Vitro Sciences, Inc. (IIVS). IIVS performed additional testing to complete parallel sets of *in vivo* and *in vitro* data and described the AMCP testing strategy in a background review document (BRD). NICEATM received an initial draft of the AMCP BRD on December 27, 2007. A formal transmittal letter followed on January 8, 2008. **Appendix A** provides a detailed timeline of the ICCVAM evaluation. The ICCVAM recommended test method protocol for each test method are provided in **Appendix B**.

The EPA and the ATWG requested that NICEATM and ICCVAM assess the scientific validity of the AMCP testing strategy as described in the AMCP BRD. The EPA and the ATWG sought to determine whether the EPA could be reasonably certain that the testing strategy would be useful for making hazard classification and labeling decisions for AMCPs.

The ICCVAM Ocular Toxicity Working Group (OTWG) worked with NICEATM in evaluating the AMCP testing strategy. Drs. João Barroso, Thomas Cole, and Valerie Zuang represented the European Centre for the Validation of Alternative Methods (ECVAM). Dr. Hajime Kojima was the liaison from the Japanese Center for the Validation of Alternative Methods (JaCVAM). On March 17, 2008, after a preliminary review of the AMCP BRD, the OTWG requested additional documents from IIVS to fill essential information gaps noted in the original submission.

On April 4, 2008, NICEATM published a request for relevant data and nominations of individuals to serve on an independent international scientific peer review panel (Panel) (73 FR 18535). The request was also sent via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholders. In response to these requests, 12 individuals or organizations nominated member to the Panel; however, no test method data were submitted.

The OTWG provided comments and requested additional information from IIVS on April 18, 2008. On June 23-24, 2008, the OTWG and ICCVAM assigned this activity a high priority after considering comments from the public and ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM).

IIVS provided a revised AMCP BRD (**Appendix C, Annex I**) and AMCP BRD Supplement (**Appendix C, Annex II**) on July 21 and October 8, 2008, respectively.

To facilitate peer review, the OTWG and NICEATM prepared a draft AMCP summary review document (SRD). The AMCP SRD summarizes the available data and information regarding the validity of each of the three *in vitro* test methods, the AMCP testing strategy, and an alternate AMCP testing strategy.

On March 31, 2009, ICCVAM announced the availability of the ICCVAM draft documents and a public Panel meeting to review the validation status of the test methods (74 FR 14556[1]). The ICCVAM draft AMCP SRD and draft test method recommendations were posted on the NICEATM–ICCVAM website. All of the information provided to the Panel and all public comments received before the Panel meeting were made available on the NICEATM–ICCVAM website.[2]

The Panel met in public session on May 19-21, 2009, to review the ICCVAM draft AMCP SRD for completeness and accuracy. The Panel then evaluated (1) the extent to which the draft AMCP SRD addressed established validation and acceptance criteria and (2) the extent to which the draft AMCP SRD supported ICCVAM's draft proposed test method recommendations. Interested stakeholders from the public commented at the Panel meeting. The Panel considered these comments, as well as those submitted previously, before concluding their deliberations. On July 13, 2009, ICCVAM posted the final report of the Panel's recommendations (**Appendix D**) on the NICEATM–ICCVAM website for public review and comment (74 FR 33444[3]).

ICCVAM provided SACATM with the draft AMCP SRD, and all public comments for discussion at their meeting on June 25–26, 2009, where public stakeholders were given another opportunity to comment.

After SACATM's meeting, ICCVAM and the OTWG considered the SACATM comments, the Panel report, and all public comments (**Appendix E**) before finalizing the ICCVAM test method evaluation report and the AMCP SRD provided in this report. As required by the ICCVAM Authorization Act, ICCVAM will make this test method evaluation report and the accompanying final SRD available to the public and to U.S. Federal agencies for consideration. The relevant U.S. Federal laws, regulations, guidelines, and recommendations for eye irritation/corrosion testing are summarized in **Appendix F**. Federal agencies must respond to ICCVAM within 180 days after receiving ICCVAM test method recommendations. Agency responses will be made available to the public on the NICEATM–ICCVAM website as they are received.

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1 http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf
2 http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm
3 http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf

2.0 ICCVAM Recommendations for the AMCP Testing Strategy

2.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

Given the limitations of the available database for the three *in vitro* test methods (i.e., BCOP, CM, and EO), there is currently insufficient data with which to adequately demonstrate that the AMCP testing strategy using these test methods can identify all four EPA ocular hazard categories.

Of the 228 AMCPs included in the validation database, none has been tested in all three *in vitro* test methods. There are a limited number of AMCPs (n=28) that have been tested in both the BCOP and EO test methods. However, of these, there is only one EPA Category II substance and only four EPA Category III substances (based on Draize rabbit eye test data). Therefore, although the performance of the alternate AMCP testing strategy using the BCOP and EO test methods appears to be useful for identifying EPA Category I substances using the BCOP test method and EPA Category IV substances using the EO test method, there is insufficient data with which to adequately demonstrate that this strategy can identify all four EPA ocular hazard categories.

Therefore, ICCVAM concludes that there are not enough data to support the AMCP testing strategy in terms of the proposed test method usefulness and limitations (i.e., the classification of substances in all four EPA ocular hazard categories). ICCVAM also concludes that there are insufficient available data on which to base definitive recommendations on the alternate AMCP testing strategy for classifying substances in all four EPA ocular hazard categories.

2.1.1 Independent Peer Review Panel Conclusions and Recommendations

The Panel concurred with ICCVAM's conclusion that there are not enough data to support the AMCP testing strategy in terms of the proposed test method usefulness and limitations (i.e., the classification of substances in all four EPA ocular hazard categories). Likewise, the Panel also concluded that there were insufficient available data on which to base definitive recommendations on the alternate AMCP testing strategy for classifying substances in all four EPA ocular hazard categories.

The Panel indicated that a retrospective evaluation of results in more than one test method can be considered adequate for the evaluation of test method performance. Retrospective studies must include an audit of the data to determine quality, comprehensiveness, and the number and severity of data errors. However, given the lack of available data for substances tested in more than one of the proposed test methods included in the strategy, the Panel concluded that any definitive recommendations should be based on prospective testing of a list of reference substances in each of the proposed *in vitro* test methods.

2.2 ICCVAM Recommendations: Test Method Protocol

The detailed test method protocols included in the AMCP BRD (**Appendix C, Annex I**) use a variety of endpoints to predict ocular irritation potential. While these test method protocols have not been adequately validated for use in the AMCP testing strategy, decision criteria have been developed to correspond to the four different categories of ocular irritation defined by the EPA hazard classification system (i.e., EPA Categories I, II, III, and IV).

Concurrent positive and negative controls should be included in each study. Additionally, ICCVAM recommends that appropriate benchmark controls should be defined for each hazard category. Periodic testing (i.e., at intervals $\leq$ 6 months) of these benchmark controls should be performed in laboratories that regularly conduct an *in vitro* testing strategy. Users should be aware that a negative study result will have ramifications on test substance results obtained in the interval between the last acceptable benchmark control study and the unacceptable benchmark control study. ICCVAM recommends using the updated ICCVAM protocols for the BCOP, CM, and EO test methods that are included as appendices to this report **(Appendix B)**. In addition, all future studies intended to further characterize the usefulness and limitations of these test methods (i.e., BCOP, CM, and EO) should be conducted using the ICCVAM recommended protocols.

2.2.1 Independent Peer Review Panel Conclusions and Recommendations

The Panel concluded that the available data supported the ICCVAM recommendations for the ocular test method procedures in terms of the proposed test method protocols.

2.3 ICCVAM Recommendations: Future Studies

Given the limitations in the validation database, a reference list of AMCPs (for which high quality Draize rabbit eye test data are available) should be tested prospectively in each of the proposed test methods (i.e., BCOP, CM, and EO) to allow for a more complete evaluation of the usefulness and limitations of the AMCP testing strategy.

The following additional recommendations are made:

- Future test methods should consider cells and tissue constructs of cornea/conjunctiva origins.
- Industry stakeholders are encouraged to provide strategies and approaches that are currently used for corporate decisions on product safety in an integrated decision strategy, including the various types of data and information and the respective qualitative and quantitative decision criteria.
- ICCVAM encourages users to provide all data that are generated from future studies, as they could be used to further characterize the usefulness and limitations of an *in vitro* testing strategy.

2.3.1 Independent Peer Review Panel Conclusions and Recommendations

The Panel concluded that additional testing would expand existing databases and could be used to optimize test method decision criteria. Additional studies recommended by the Panel are reflected in the ICCVAM recommendations detailed above. The Panel also concluded that additional studies should not focus on the use of the EO test method alone because it considered the use of an *in vitro* testing strategy more promising.

2.4 ICCVAM Recommendations: Performance Standards

Based on the available data and associated performance described in **Sections 3.2** and **3.4**, ICCVAM recommends that the development of performance standards for the AMCP testing strategy is not warranted at this time.

2.4.1 Independent Peer Review Panel Conclusions and Recommendations

The Panel concluded that the development of performance standards for the AMCP testing strategy was not warranted at this time.

3.0 Validation Status of the AMCP Testing Strategy

The information in the ICCVAM final AMCP summary review document (**Appendix C**) is summarized below. The SRD reviews the available data and information for the AMCP testing strategy. It describes the current validation status of the AMCP testing strategy, including what is known about its reliability and accuracy, the scope of the substances tested, and standardized protocols used for the validation study.

3.1 Test Method Description

3.1.1 AMCP Testing Strategy

The AMCP testing strategy (**Figure 3-1**) proposed in the AMCP BRD (**Appendix C, Annex I**) comprises three *in vitro* test methods: the BCOP, CM, and EO. Each test method includes decision criteria developed to correspond to the four ocular irritation categories defined in the EPA hazard classification system. The BCOP, CM, and EO test methods use a variety of endpoints to predict ocular irritation potential.

The two primary endpoints for the BCOP test method are the extent of corneal opacity and the permeability. Both are measured and used to calculate an *in vitro* irritancy score (IVIS).[4]

- IVIS $\geq$ 75 = EPA Category I
- IVIS $\geq$ 25 and < 75 = EPA Category II
- IVIS < 25 = EPA Category III

Because the data points from EPA Category III and Category IV overlap and it's impossible to assign a cutoff value, the AMCP BRD does not propose BCOP decision criteria for EPA Category IV. Histopathology evaluation of the affected tissue is an optional endpoint.

The endpoint for the CM test method is the estimated concentration of a test substance needed to reduce the basal metabolic rate of L929 cells by 50% (MRD_{50}).

- MRD_{50} < 2 mg/mL = EPA Category I
- MRD_{50} ≥ 2mg/mL and < 80 mg/mL = EPA Category III
- MRD_{50} ≥ 80 mg/mL = EPA Category IV

The AMCP BRD does not propose CM decision criteria for EPA Category II because the data points from EPA Category I and Category II overlap making it impossible to assign a cutoff value.

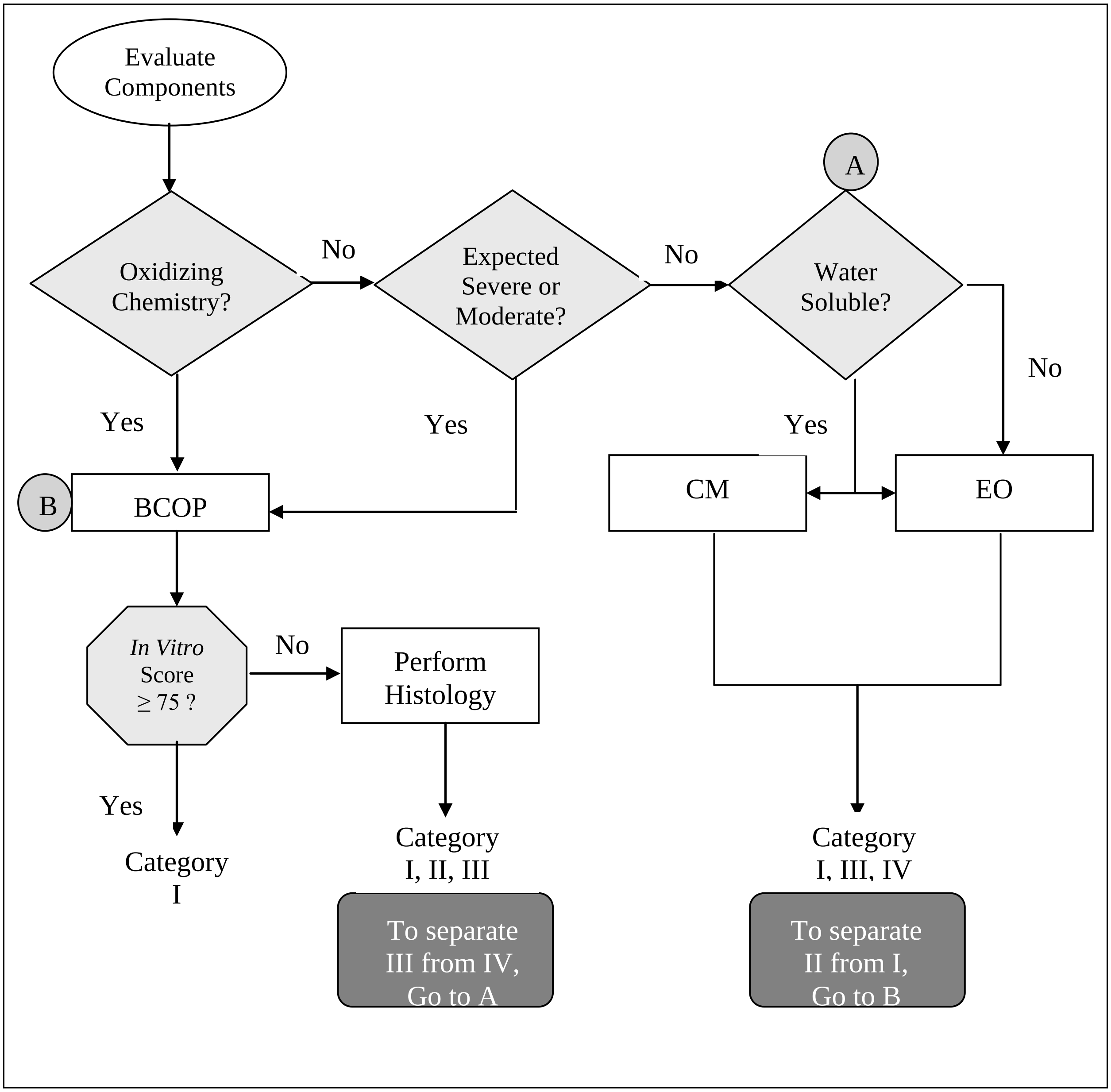
The endpoint for the EO test method is the time needed to reduce cell viability by 50% (ET_{50}).

- ET_{50} < 4 min = EPA Category I
- ET_{50} ≥ 4 min and < 70 min = EPA Category III
- ET_{50} ≥ 70 mg/mL = EPA Category IV

The AMCP BRD does not propose EO decision criteria for EPA Category II because the database includes only one EPA Category II substance.

[4] The *in vitro* irritancy score (IVIS) is calculated as the sum of the mean corrected opacity value (± standard deviation [SD]) and 15 times the mean corrected permeability value (OD_{490} units ± SD).

Figure 3-1 Combining the BCOP, CM, and EO Test Methods into a Testing Strategy: AMCP Testing Strategy



In the AMCP testing strategy (**Figure 3-1**), the first test method used depends on knowledge of the chemical properties of the test substance. If the test substance is an oxidizer, which suggests that it will be an ocular corrosive/severe irritant, it is first tested in the BCOP test method. As noted above, test substances that produce an IVIS ≥ 75 would be classified as EPA Category I. If a test substance produces an IVIS < 75, further assessment using histopathology evaluation can determine whether it meets the criteria for classification as EPA Category I, II, or III.

To determine whether the test substance is EPA Category III or IV, the test substance is subsequently tested in either the CM or EO test method. Selection of the CM or EO test method depends on the water solubility of the test substance. Water-soluble substances can be tested in either the CM test method or the EO test method, but water-insoluble substances must be tested in the EO test method to determine their final hazard classification.

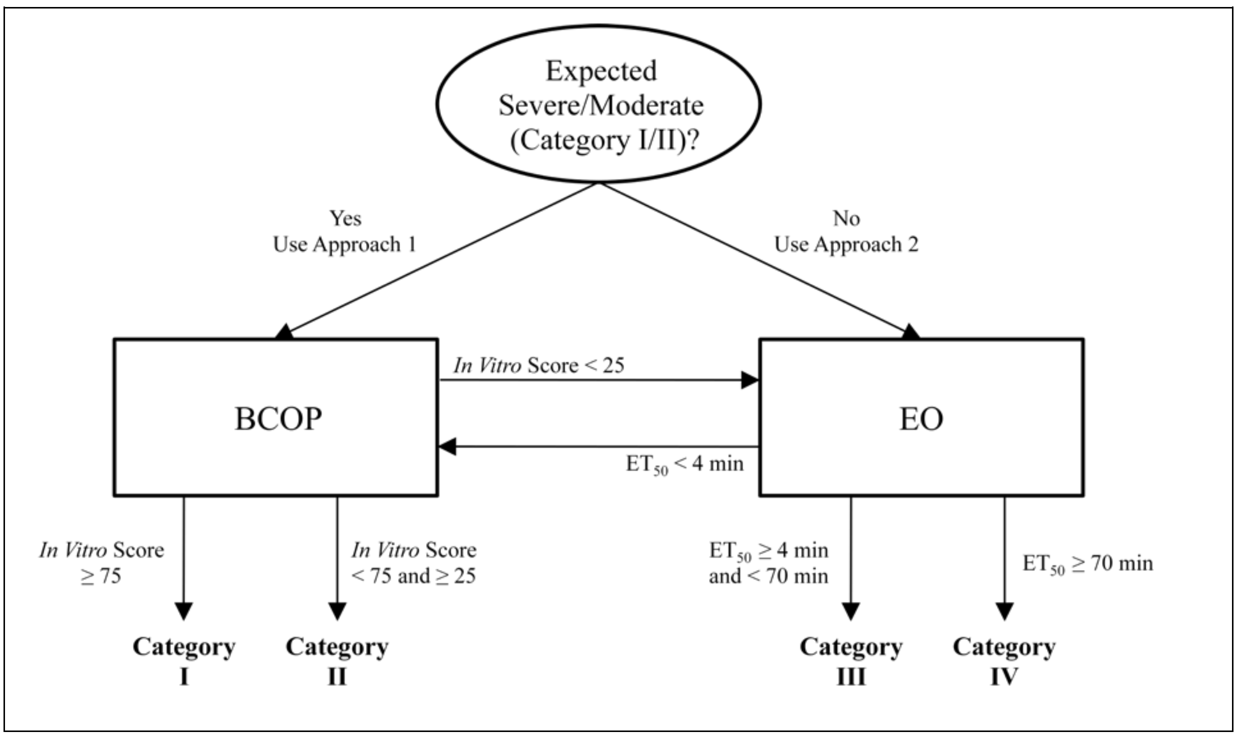
3.1.2 Combining the BCOP and EO Test Methods into a Testing Strategy: Alternate AMCP Testing Strategy

None of the 228 substances has been tested in all three of the *in vitro* test methods included in the AMCP testing strategy. There were also concerns about the validation status of the low volume eye test (LVET), which was used as the *in vivo* reference test method for all of the CM test method data. Therefore, ICCVAM evaluated an alternate AMCP testing strategy (**Figure 3-2**) that includes only the BCOP and EO test methods. In the alternate AMCP testing strategy, the BCOP test method would be used to identify EPA Category I or II substances, and the EO test method would be used to identify EPA Category III or IV substances.

ICCVAM evaluated two approaches in the alternate AMCP testing strategy: (1) test in the BCOP test method first and then in the EO test method or (2) test in the EO test method first and then in the BCOP test method. In the first proposed approach, the BCOP test method would classify all EPA Category I and II substances. The EO test method would then classify all other substances as either EPA Category III or IV.

In the second proposed approach, the EO test method would classify all EPA Category III and IV substances. All other substances would then be tested in the BCOP test method and classified as either EPA Category I or II.

Figure 3-2 Combining the BCOP and EO Test Methods into a Testing Strategy: Alternate AMCP Testing Strategy



3.2 Validation Database

3.2.1 Rationale for the Substances or Products Included in the AMCP Testing Strategy

The validation database for the AMCP BRD included 228 substances (**Appendix C, Annex I**). These include 68 substances tested in the BCOP test method, 105 substances tested in the CM test method, and 55 substances tested in the EO test method. None of the 228 substances has been tested in all three of the proposed *in vitro* test methods (i.e., BCOP, CM, and EO). It should be noted that, according to the submitter, “a minimum 28 of the materials are EPA registered antimicrobial cleaning products, with eight additional materials being in-use dilutions of concentrates which are EPA registered” (Rodger Curren, personal communication).

The distribution of product categories differed for each test method (**Table 3-1**). Most of the 105 substances tested in CM test method are surfactants (78% [82/105]) and solvents (17% [18/105]). The 68 substances tested in the BCOP test method and the 55 substances tested in the EO test method are relatively equally distributed among alkalis, oxidizers, solvents, and surfactants (approximately 20% to 30% each).

Table 3-1 Distribution of Product Categories Evaluated in the AMCP Testing Strategy

Product Categories	Number of Substances Tested Per Test Method			
	Cytosensor Microphysiometer	EpiOcular™	BCOP	Total
Solvents	18	10	12	39
Oxidizers	0	13	16	33
Surfactants	82	17	18	114
Acids	1	2	7	10
Bases	4	11	14	29
Others	0	2	1	3
Total	105	55	68	228

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability.

3.2.2 Rationale for the Substances or Products Included in the Alternate AMCP Testing Strategy

Only 28 substances tested in both the BCOP and EO and test methods were also tested in the Draize rabbit eye test. Therefore, ICCVAM limited its evaluation of the alternate AMCP testing strategy to these 28 substances. These substances included five surfactants, two acids, ten alkalis, four oxidizers, six solvents, and one “other” (or nonspecified) (**Table 3-2**). The Draize rabbit eye test classified only one as EPA Category II and only four as EPA Category III (**Table 3-2**).

Table 3-2 Distribution of Product Categories Evaluated in the Alternate AMCP Testing Strategy

Product Category	Number of Products Tested	*In Vivo* Draize Classification — EPA			
		I	II	III	IV
Surfactant	5	0	0	2	3
Acid	2	0	0	1	1
Alkali	10	9	1	0	0
Oxidizer	4	3	0	0	1
Solvent	6	2	0	1	3
Other	1	0	0	0	1
Total	28	14	1	4	9

Abbreviations: AMCP = antimicrobial cleaning product; EPA = U.S. Environmental Protection Agency

3.3 Reference Test Method Data

Varied test method protocols were used to generate the *in vivo* reference data for the 228 substances tested in the AMCP testing strategy (**Table 3-3**). Of the 68 substances tested in the BCOP test method, 85% (58/68) were tested using the traditional Draize rabbit eye test protocol (OECD 2002). Another 12% (8/68) were tested in a nontraditional protocol (i.e., application of 30 µL instead of 100 µL or application as an aerosol spray). The remaining 3% (2/68) were tested in the LVET. The LVET is a modification of the Draize rabbit eye test that involves application of 10 µL of the test substance directly to the corneal surface rather than application of 100 µL of the test substance into the conjunctival sac. All 105 substances tested in the CM test method were tested in the LVET. Of the 55 substances tested in EO test method, 55% (30/55) were tested in the Draize rabbit eye test. Forty-five percent (25/55) were tested in the LVET. None of the 228 substances was tested in both the Draize rabbit eye test and the LVET.

Table 3-3 Distribution of *In Vivo* Reference Data

Test Method	Number of AMCPs Tested	LVET	Draize[1]		LVET and Draize
			Traditional	Nontraditional	
BCOP	68	2	58[2]	8[3]	0
CM	105	105	0	0	0
EO	55	25	30	0	0
Total	228	132	88	8	0

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; CM = Cytosensor Microphysiometer; EO = EpiOcular™; LVET = low volume eye test

[1] The traditional Draize protocol involves instillation of 0.1 mL of test substance into the conjunctival sac of a rabbit eye. The nontraditional Draize protocol doses with 0.03 mL of test substance into the conjunctival sac of a rabbit eye.

[2] The dose volume for one substance was not provided. It was included in the traditional Draize total.

[3] One of the substances was evaluated as an aerosol sprayed directly on the cornea for one second.

The alternate AMCP testing strategy is based on the results for the 28 substances that (1) were tested in both the BCOP and the EO test methods (see **Table 3-2**) and (2) were also tested in the Draize rabbit eye test and qualified for assignment of an EPA ocular hazard classification.

3.4 Test Method Accuracy

3.4.1 The Bovine Corneal Opacity and Permeability Test Method

The accuracy of the overall EPA classification was 55% (36/66) (**Table 3-4**) in the validation database of 66 substances tested in both the BCOP test method and the Draize rabbit eye test. Of these, the BCOP test method had only 60% (3/5) accuracy in identifying EPA Category II substances and 50% (6/12) accuracy in identifying EPA Category III substances. The BCOP correctly identified 90% (27/30) of the substances classified as EPA Category I by the Draize rabbit eye test.

Among the three EPA Category I substances that were underpredicted as EPA Category II by the BCOP test method, two were oxidizers and one was a base. It should be noted that the base would be correctly identified if the decision criteria were IVIS ≥ 55.1, as recommended in the 2006 ICCVAM BRD (ICCVAM 2006a), instead of IVIS ≥ 75 as proposed in the AMCP BRD (**Appendix C, Annex I**). However, such a change in decision criteria would also result in the overprediction of two EPA Category II substances (one oxidizer and one acid) and one EPA Category III substance (a base) as EPA Category I.

Among the EPA Category II substances that were incorrectly identified by the BCOP test method, one (a base) was underclassified as EPA Category III. One (an oxidizer) was overclassified as EPA Category I. The six EPA Category III substances incorrectly identified by the BCOP test method were overclassified as either EPA Category II (one solvent, one base, and one surfactant) or EPA Category I (two oxidizers and one base). Because the AMCP BRD does not propose BCOP decision criteria for EPA Category IV, the BCOP test method overpredicted 19 substances. The BCOP identified two as EPA Category II (both solvents) and 17 as EPA Category III (8 surfactants, 3 solvents, 3 acids, one base, one oxidizer, and one "other").

To assess the use of histopathology evaluation, BCOP test method data with histopathology evaluation were compared to BCOP test method data only. Seventeen substances had BCOP test method data with histopathology evaluation. As noted in **Table 3-5**, the overall accuracy for EPA hazard classifications (i.e., EPA Category I, II, III, and IV) was reduced from 41% (7/17) to 35% (6/17) with histopathology evaluation. Using histopathology evaluation with the BCOP test method removed one of the EPA Category I false negatives, but added three EPA Category II false positives.

Table 3-4 Performance of AMCPs in the Bovine Corneal Opacity and Permeability, Cytosensor Microphysiometer, and EpiOcular™ Test Methods Compared to the Draize Rabbit Eye Test or the Low Volume Eye Test as Reported in the AMCP BRD Using the EPA Ocular Hazard Classification System

***In Vitro* Test Method**	***In Vivo* Test Method**	**Overall Classification**	**Performance of the *In Vitro* Test Method Compared to the *In Vivo* Reference Test Method Using the EPA Ocular Hazard Classification System**									
			Category I		**Category II**			**Category III**			**Category IV**	
			Actual	**Under**	**Over**	**Actual**	**Under**	**Over**	**Actual**	**Under**	**Over**	**Actual**
BCOP[1]	Draize	55% (36/66)	90% (27/30)	10% (3/30)	20% (1/5)	60% (3/5)	20% (1/5)	50% (6/12)	50% (6/12)	0% (0/12)	100% (19/19)	0% (0/19)
CM[2]	LVET	30% (32/108)	100% (9/9)	0% (0/9)	100% (11/11)	0% (0/11)	0% (0/11)	67% (40/60)	33% (20/60)	0% (0/60)	89% (25/28)	11% (3/28)
EO[3]	Draize	76% (22/29)	100% (15/15)	0% (0/15)	0% (0/1)	0% (0/1)	100% (1/1)	25% (1/4)	75% (3/4)	0% (0/4)	56% (5/9)	44% (4/9)
EO[4]	LVET	44% (11/25)	100% (3/3)	0% (0/3)	100% (1/1)	0% (0/1)	0% (0/1)	33% (4/12)	67% (8/12)	0% (0/12)	100% (9/9)	0% (0/9)

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; CM = Cytosensor Microphysiometer; EO = EpiOcular™; EPA = U.S. Environmental Protection Agency; ET_{50} = estimated time to decrease keratinocyte viability in the EO test method by 50%; IVIS = *in vitro* irritancy score; LVET = low volume eye test; MRD_{50} = concentration of test substance that decreases the metabolic rate by 50% determined by a plot of the concentration-response curve.

[1] Classification of the BCOP data was based on IVIS ≥ 75 = EPA Category I; IVIS ≥ 25 and < 75 = EPA Category II; IVIS < 25 = EPA Category III. The BCOP test method was not proposed to identify EPA Category IV. All BCOP classifications, including high-solvent substances, used a 10-minute exposure time. The database comprised 66 substances tested in both the BCOP test method and the Draize rabbit eye test.

[2] Classification of the CM data was based on MRD_{50} < 2 mg/mL = EPA Category I; MRD_{50} ≥ 2mg/mL and < 80 mg/mL = EPA Category III; MRD_{50} ≥ 80 mg/mL = EPA Category IV. The CM test method was not proposed to identify EPA Category II. The database consisted of 108 substances tested in both the CM test method and in the LVET (105 different substances because three substances were tested twice).

[3] Classification of the EO data was based on ET_{50} < 4 min = EPA Category I; ET_{50} ≥ 4 min and <70 min = EPA Category III; ET_{50} ≥ 70 min = EPA Category IV. The EO test method was not proposed to identify EPA Category II. The database consisted of 29 substances tested in both the EO test method and the Draize rabbit eye test that qualified for EPA hazard classification (i.e., one substance producing a Draize score greater than 1 was not evaluated through day 21 as required by EPA).

[4] Classification of the EO data was based on ET_{50} < 4 min = EPA Category I; ET_{50} ≥ 4 min and < 70 min = EPA Category III; ET_{50} ≥ 70 min = EPA Category IV. The EO test method was not proposed to identify Category II. The database consisted of 25 substances tested in both the EO test method and the LVET.

Table 3-5 Comparison of the BCOP Test Method and the BCOP Test Method Using Histopathology Evaluation

EPA	Overall Classification	Draize Test									
		Category I		Category II			Category III			Category IV[1]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
BCOP[2] only	41% (7/17)	50% (3/6)	50% (3/6)	0% (0/4)	75% (3/4)	25% (1/4)	75% (3/4)	25% (1/4)	0% (0/4)	100% (3/3)	0% (0/3)
BCOP[2] with histopathology	35% (6/17)	67% (4/6)	33% (2/6)	75% (3/4)	25% (1/4)	0% (0/4)	75% (3/4)	25% (1/4)	0% (0/4)	100% (3/3)	0% (0/3)

Abbreviations: BCOP = bovine corneal opacity and permeability

[1] The BCOP test method decision criteria do not propose to identify EPA Category IV substances.

[2] The BCOP test method was based on the use of decision criteria with a cutoff for corrosives/severe irritants of ≥ 75 tested with a 10-minute exposure time.

3.4.2 The Cytosensor Microphysiometer Test Method

The validation database included 108 substances tested in both the CM test method and the LVET (**Table 3-4**). Accuracy of the overall EPA classification (i.e., EPA Category I, II, III, and IV) was 30% (32/108). It should be noted that the database consisted of 105 unique substances because three substances were tested twice. The CM overclassified the majority of substances classified by the LVET as EPA Category II, III, and IV. Overclassification included 100% (11/11) of the EPA Category II substances, 67% (40/60) of the EPA Category III substances, and 89% (25/28) of the EPA Category IV substances. Among the 25 overclassified EPA Category IV substances, 16% (4/25; all surfactants) were classified by the CM test method as EPA Category I, and 84% (21/25; 6 solvents, 2 bases, and 13 surfactants) were classified by the CM test method as EPA Category III. Because decision criteria for the CM test method are not proposed in the AMCP BRD for EPA Category II, all EPA Category II or III substances that were overclassified by the CM test method were classified as EPA Category I. All but one of the 40 EPA Category III substances (a solvent) that were overclassified by the CM test method were surfactants. All 11 of EPA Category II substances that were overclassified by the CM test method were surfactants. All nine of the EPA Category I substances (all surfactants) were correctly identified. None of the irritant categories (i.e., EPA Category I, II, or III) were underpredicted by the CM test method.

3.4.3 The EpiOcular Test Method

Among the 55 substances tested in the EO test method (**Table 3-4**), 30 were also tested in the Draize rabbit eye test (29 qualified for EPA hazard classification [i.e., one substance producing a Draize score greater than 1 was not evaluated through day 21 as required by EPA]), and 25 were tested in the LVET. Based on the database of 29 substances tested in both the EO test method and the Draize rabbit eye test, accuracy of the overall EPA classification (i.e., EPA Category I, II, III, and IV) was 76% (22/29). Among the four EPA Category III substances, 75% (3/4) were correctly identified by the EO test method. The one substance incorrectly identified (a base) was overclassified as EPA Category I. Among the nine EPA Category IV substances, 44% (4/9) were correctly identified. Four of the five incorrectly identified substances were overclassified as EPA Category III (two solvents, one acid, and one surfactant). The remaining substance (a surfactant) was overclassified as EPA Category I. The EO test method correctly identified all of the EPA Category I substances (15/15, including 12 bases, two solvents, and one “other”).

The EO test method correctly classified 44% (11/25) of the 25 substances tested in both the EO test method and the LVET (**Table 3-4**). Among the 12 substances classified by the LVET as EPA Category III, the EO test method correctly identified 67% (8/12). The four substances incorrectly identified (two surfactants and two oxidizers) were overclassified as EPA Category I. The EO test method did not correctly identify any of the nine EPA Category IV substances. Forty-four percent (4/9: three surfactants and one solvent) were overclassified as EPA Category III, and 56% (5/9: three oxidizers and two solvents) were overclassified as EPA Category I. The EO test method correctly identified all of the EPA Category I substances (3/3: two oxidizers and one surfactant).

3.4.4 AMCP Testing Strategy

Table 3-4 summarizes **t**he performance of each test method included in the AMCP testing strategy. None of the 228 substances included in the AMCP BRD was tested in all three of the proposed *in vitro* test methods. Therefore, no data are available with which to characterize the actual performance of the AMCP testing strategy that includes all three test methods: the BCOP, CM, and EO.

3.4.5 Alternate AMCP Testing Strategy

Twenty-eight substances with Draize rabbit eye test data were tested in both the BCOP and EO test methods. In the alternate AMCP testing strategy, the BCOP test method is intended to identify only EPA Category I and II substances. The EO test method is intended to identify only EPA Category III and IV substances. As described in **Section 3.1.2**, the alternate AMCP testing strategy could follow one of two approaches. The performance of the alternate AMCP testing strategy was the same (**Table 3-6**) regardless of which approach was used.

The alternate AMCP testing strategy correctly classified 79% (22/28) of the substances. Among these, it correctly identified all of the EPA Category I substances (14/14), all of the EPA Category III substances (4/4), and 44% (4/9) of the EPA Category IV substances. The one EPA Category II substance was underpredicted as EPA Category III. Furthermore, classification of the BCOP data using either the decision criteria in the AMCP BRD (**Appendix C, Annex I**) (IVIS ≥ 75 for EPA Category I) or in the 2006 ICCVAM BRD (IVIS ≥ 55 for EPA Category I) yielded identical results. All BCOP classifications, including high-solvent substances, used a 10-minute exposure time. When using 3-minute data for high solvents, the overall classification was 74% (17/23). Five high-solvent substances did not have 3-minute data; therefore, they cannot be considered in this analysis. It should be noted that, based on this limited database of 28 substances, the performance of the EO test method alone is the same as that of the alternate AMCP testing strategy.

Table 3-6 Performance of AMCPs Tested in Both the BCOP and EO Test Methods

EPA	Overall Classification	Draize									
		I		II			III			IV	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Approach 1	79% (22/28)	100% (14/14)	0% (0/14)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/4)	100% (4/4)	0% (0/4)	56% (5/9)	44% (4/9)
Approach 2	79% (22/28)	100% (14/14)	0% (0/14)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/4)	100% (4/4)	0% (0/4)	56% (5/9)	44% (4/9)

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; EO = EpiOcular™; EPA = U.S. Environmental Protection Agency.

Approach 1 = Test in the BCOP test method first to classify as either EPA Category I or II and then in the EO test method to identify EPA Category III and IV.

Approach 2 = Test in the EO test method first to classify as either EPA Category III or IV and then in the BCOP test method to identify EPA Category I and II.

3.5 Test Method Reliability

3.5.1 The Bovine Corneal Opacity and Permeability Test Method

Intralaboratory repeatability is determined by comparing within-experiment runs of a test substance. Intralaboratory repeatability for the BCOP test method was quantitatively determined for 67 AMCPs (four substances have repeat tests) as the mean %CV for opacity, permeability, and IVIS (AMCP BRD; **Appendix C, Annex I**). Because a very low IVIS significantly affects %CV, the overall mean %CV calculations did not include substances with an IVIS ≤ 10 (arbitrarily set in the AMCP BRD). The overall mean %CVs for opacity, permeability, and IVIS were 21%, 25%, and 18%, respectively.

These 67 test substances, tested in a total of 75 runs, were also qualitatively evaluated for their concordance using the EPA (EPA 2003) and GHS (UN 2007) ocular hazard classification systems (AMCP BRD Supplement; **Appendix C, Annex II**). For the EPA and GHS classification systems, there was 100% agreement among the corneas in 63 of the 75 runs (84%). There was 67% agreement in 11 of 75 runs (15%) and 60% agreement in one of 75 runs (1%). Of the 12 runs in which the test corneas were not in 100% agreement, seven had reactive chemistries, two were alkalis, two were surfactants, and one was an acid.

Intralaboratory repeatability for the BCOP test method has been quantitatively determined for non-AMCPs predicted as ocular corrosives/severe irritants in the BCOP test method in three studies (16-52 substances) (ICCVAM 2006a). The mean %CV for IVIS ranged from 39% to 71%.

Intralaboratory reproducibility is determined by comparing between-experiment runs of a test substance. For the BCOP test method, intralaboratory reproducibility was quantitatively determined for five AMCPs. For these five substances (2–6 experiments), the mean %CV for IVIS was 20% (see Section 7.3 of the AMCP BRD, **Appendix C, Annex I**).

These test substances were also qualitatively evaluated for their concordance using the EPA (EPA 2003) and GHS (UN 2007) ocular hazard classification systems (see Section 3.2 of the AMCP BRD Supplement, **Appendix C, Annex II**). The five test substances had 100% agreement in the EPA and GHS classification systems.

Intralaboratory reproducibility for the BCOP test method has been quantitatively determined for non-AMCPs predicted as ocular corrosives/severe irritants in the BCOP test method (ICCVAM 2006a). In one study composed of 25 surfactant-based personal care cleaning formulations, the mean %CV for permeability values was 33%. In the second study, the mean %CV for IVIS ranged from 13% to 15% for 16 test substances.

Interlaboratory reproducibility is determined by comparing between-laboratory runs of a test substance. Interlaboratory reproducibility for the BCOP test method could not be determined specifically for the AMCPs presented in the AMCP BRD (**Appendix C, Annex I**) because only one laboratory conducted the testing.

Interlaboratory reproducibility for the BCOP test method has been quantitatively determined for non-AMCPs predicted as ocular corrosives/severe irritants in the BCOP test method (ICCVAM 2006a). In three studies (3–12 laboratories each), the mean %CV for IVIS ranged from 25% to 36%. The study results were also qualitatively evaluated for their concordance using the EPA (EPA 2003), EU (EU 2001), and GHS (UN 2007) ocular hazard classification and labeling systems (ICCVAM 2006a).

3.5.2 The Cytosensor Microphysiometer Test Method

Reliability for the CM test method could not be evaluated specifically for AMCPs due to insufficient data. However, quantitative evaluations of reliability were conducted based on non-AMCPs tested in the CM test method (**Appendix C, Annexes I** and **II**).

Intralaboratory repeatability for the CM test method was quantitatively evaluated for non-AMCPs in seven studies of one to 35 test substances each (**Appendix C, Annexes I** and **II**). The mean % coefficient of variation (CV) for MRD_{50} values for all materials tested, including surfactant and nonsurfactant materials, ranged from 6% to 25%.

Intralaboratory reproducibility was quantitatively determined for non-AMCPs in one laboratory (16 substances) (**Appendix C, Annex I**). The mean %CV for MRD_{50} values for all materials tested, including surfactant and nonsurfactant materials, was 25%.

Interlaboratory reproducibility for the CM test method was quantitatively determined for non-AMCPs in two studies (2–4 laboratories each) (**Appendix C, Annex I** and **II**). The mean %CV for MRD_{50} values for all materials tested, including surfactant and nonsurfactant materials, ranged from 17% to 51%. Nonsurfactant materials had a higher mean %CV in each study.

3.5.3 The EpiOcular Test Method

Intralaboratory repeatability for the EO test method was quantitatively determined specifically for a subset of 15 AMCPs presented in the AMCP BRD (**Appendix C, Annex I**). The mean %CV for ET_{50} values ranged from 0% to 62%.

To evaluate concordance using the EPA (EPA 2003) and GHS (UN 2007) ocular hazard classification systems (AMCP BRD Supplement, **Appendix C, Annex II**), qualitative analyses were conducted for three AMCPs that were tested more than once at IIVS. There was 100% agreement for all three AMCPs in both classification systems.

Intralaboratory reproducibility for the EO test method was quantitatively determined from repeat testing of a single substance (0.3% Triton X-100). Data were presented as combined data from MatTek Corporation and IIVS (9-year period) and from IIVS only (8-year period). The mean %CV for ET_{50} values was 21% and 22%, respectively.

Interlaboratory reproducibility for the EO test method cannot be determined specifically for the AMCPs presented in the AMCP BRD (**Appendix C, Annex I**) because only one laboratory conducted the testing. However, interlaboratory reproducibility for the EO test method was quantitatively determined for non-AMCPs in a phased validation study of surfactants and surfactant-containing products. The validation study is summarized in the AMCP BRD (**Appendix C, Annex I**). The mean %CVs ranged from 12% to 18%. However, it should be noted that this evaluation was based on an EO protocol that uses relative percent viability to assign an irritancy classification (irritant or nonirritant). It did not use a calculated ET_{50} value to predict the EPA ocular hazard category. This protocol is included in the AMCP BRD.

These test substances were also qualitatively evaluated for their concordance using the EPA (EPA 2003) and GHS (UN 2007) ocular hazard classification systems (AMCP BRD Supplement; **Appendix C, Annex II**). Using either the EPA or GHS classification systems in Phase II of the validation study, there was 100% agreement for 14/19 (74%) substances, 75% agreement for 2/19 (11%) substances, and 50% agreement for 3/19 (16%) substances among four laboratories. In Phase III of the validation study using the EPA or GHS ocular hazard classification systems, there was 100% agreement for 51/54 (94%) substances and 0% agreement for 3/54 (6%) substances in two laboratories.

3.6 Animal Welfare Considerations: Reduction, Refinement, and Replacement

The AMCP testing strategy proposed in the AMCP BRD is a non-animal approach for classifying and labeling AMCPs, as is the alternate AMCP testing strategy.

Bovine eyes used in the BCOP test method are obtained post mortem from animals that are being used for food. The CM test method uses L929 cells, a commercially available mouse cell line. The EO test method uses primary human keratinocytes obtained from human donors during routine surgical procedures.

4.0 ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process incorporates a high level of transparency. This process is designed to provide numerous opportunities for stakeholder involvement, including submitting written public comments and providing oral comments at ICCVAM independent peer review panel meetings and SACATM meetings. **Table 4-1** lists the nine opportunities for public comments during the ICCVAM evaluation of the validation status of alternative ocular safety testing methods and approaches. The number of public comments received is also indicated. Thirty-seven comments were submitted. Comments received in response to or related to *Federal Register* notices (**Appendix E**) are also available on the NICEATM-ICCVAM website.[5] The following sections, delineated by *Federal Register* notice, briefly discuss the public comments received.

Table 4-1 Opportunities for Public Comments

Opportunities for Public Comments	Date	Number of Public Comments Received
70 FR 13512: Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel	March 21, 2005	0
72 FR 26396: Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing	May 9, 2007	1
72 FR 31582: Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods	June 7, 2007	0
73 FR 18535: Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data	April 4, 2008	12
74 FR 14556: Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments	March 31, 2009	8
74 FR 19562: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)	April 29, 2009	2
Independent Scientific Peer Review Panel Meeting: Alternative Ocular Safety Testing Methods	May 19–21, 2009	12
SACATM Meeting, Arlington Hilton, Arlington, VA	June 25–26, 2009	2
74 FR 33444: Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments	July 13, 2009	0

[5] Available at http://ntp-apps.niehs.nih.gov/iccvambp/searchPubCom.cfm

4.1 Public Comments in Response to 70 FR 13512 (March 21, 2005) Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of AMCP Formulations; Request for Nominations for an Independent Expert Panel

NICEATM requested (1) submission of data that would assist in evaluating the validation status of non-animal methods and approaches used for determining the skin and eye irritation potential of AMCP formulations to meet regulatory hazard classification and labeling purposes and (2) nominations of expert scientists to serve as members of an independent peer review panel.

No data or nominations were received in response to this *Federal Register* notice.

4.2 Public Comments in Response to 72 FR 26396 (May 9, 2007) Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

NICEATM requested submission of (1) data and information on the use of topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during eye irritation testing and (2) information about other procedures and strategies that may reduce or eliminate pain and distress associated with *in vivo* eye irritation methods.

In response to this *Federal Register* notice, NICEATM received one comment. This comment was not relevant to the AMCP testing strategy or the three *in vitro* test methods (i.e., BCOP, CM, and EO) included in the AMCP testing strategy.

4.3 Public Comments in Response to 72 FR 31582 (June 7, 2007) Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Test Methods

NICEATM requested data on substances tested for ocular irritancy in humans, rabbits, and/or *in vitro* to be used to:

- Review the state of the science in regard to the availability of accurate and reliable *in vitro* test methods for assessing the range of potential ocular irritation activity, including whether ocular damage is reversible or not
- Expand NICEATM's high-quality ocular toxicity database. *In vitro* test methods for which data are sought include but are not limited to (1) the bovine corneal opacity and permeability test, (2) the isolated rabbit eye test, (3) the isolated chicken eye test, and (4) the hen's egg test–chorioallantoic membrane.

No data or information was received in response to this *Federal Register* notice.

4.4 Public Comments in Response to 73 FR 18535 (April 4, 2008) Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products: Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

NICEATM requested the following:

- Nominations of expert scientists to serve as members of an independent peer review panel
- Submission of relevant data and information on AMCPs or related substances obtained from (1) human testing or experience, including reports from accidental exposures, and (2) rabbit testing using the standard eye test or the LVET

- *In vitro* ocular safety test methods such as the bovine corneal opacity and permeability test method, the Cytosensor Microphysiometer test method, and the EpiOcular test method, including data supporting the accuracy and reproducibility of these methods

In response to this *Federal Register* notice, NICEATM received 12 comments, including nominations of 20 potential panelists. The nominees were included in the database of experts from which the Panel was selected. No additional data were received.

4.5 Public Comments in Response to 74 FR 14556 (March 31, 2009) Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents; Request for Comments

NICEATM requested public comments on the draft BRDs, SRDs, and draft ICCVAM test method recommendations that were provided to an independent scientific peer review panel meeting (May 19–21, 2009). These documents summarized the current validation status of several test methods and testing strategies for identifying potential ocular irritants. The test methods and testing strategies included the following:

- A testing strategy that proposes the use of three *in vitro* test methods to assess the eye irritation potential of AMCPs
- Four *in vitro* test methods for identifying moderate (EPA Category II, UN Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Category 2A) and mild (EPA Category III, GHS Category 2B) ocular irritants and substances not classified as ocular irritants (EPA Category IV, GHS Not Classified)
- The *in vivo* LVET
- A proposal for the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid and minimize pain and distress during *in vivo* ocular safety testing

NICEATM received 20 comments in response to this *Federal Register* notice. Eight written comments were received before the Panel meeting, and 12 oral comments were provided at the Panel meeting.

<u>Public Responses (written)</u>

Two of the written comments were related to the AMCP testing strategy or one of the three *in vitro* test methods (i.e., BCOP, CM, and EO) included in the AMCP testing strategy.

Comment:
One commenter acknowledged that replacement of the Draize rabbit eye test will require combinations of *in vitro* test methods and welcomed further discussions to develop these approaches, in particular in the context of the recently established International Cooperation on Alternative Test Methods (ICATM).

ICCVAM Response:
ICCVAM is fully committed to ICATM and welcomes any discussions that would promote harmonization of approaches for validation of *in vitro* test methods. ICCVAM is working to identify integrated testing strategies that could be applied to ocular toxicity testing.

Comment:
One commenter provided comments to support the value of the EO test method and outlined a proposal for an improved testing strategy for use of the BCOP and EO test methods for determination of EPA hazard classification of AMCPs. Specifically, the commenter summarized data from the AMCP SRD to indicate that the EO test method can identify EPA Categories I, II, or IV as a stand-

alone test method and that combining the BCOP and EO test methods did not provide any benefit to results obtained with the EO test method alone.

ICCVAM Response:
As noted in **Section 2.3**, ICCVAM recommends that a reference list of AMCPs (for which high-quality Draize rabbit eye test data are available) should be tested in each of the proposed test methods (i.e., BCOP, CM, and EO) to allow more complete evaluation of the usefulness and limitations of an *in vitro* testing strategy. The Panel agreed with the recommendation, having concluded that additional studies should not focus on the use of the EO test method alone. The Panel considered the use of an *in vitro* testing strategy more promising.

<u>Public Responses, Oral</u>
Twelve oral public comments were provided at the Panel meeting (May 19-21, 2009). Seven of these comments were related to the AMCP testing strategy or one of the three *in vitro* test methods (i.e., BCOP, CM, and EO) included in the AMCP testing strategy.

Comment:
One commenter indicated that the performance of the BCOP test method was unlikely to improve based on the lack of reproducibility with the Draize rabbit eye test in the mild and moderate categories. The commenter stated that results from Weil and Scala (1971) show that the extremes (i.e., corrosives/severe irritants and substances not labeled as irritants) are reproducible, but the mild and moderate levels of ocular irritation are highly variable. The commenter referenced the AMCP BRD, which includes an analysis of the impact on the ocular hazard category when the results of a six-rabbit Draize test are randomly sampled for a 3-rabbit test.

ICCVAM Response:
The Draize rabbit eye test (Draize et al. 1944) has a long history of demonstrated protection of public health; therefore, U.S. and international regulatory agencies currently use this test to identify potential ocular hazards. Alternatives are accepted only when they demonstrate the ability to provide equal or better protection than the reference test method. Given the uncertainty of the results associated with the BCOP test method for substances in the mild/moderate irritancy range, the BCOP test method cannot be considered a complete replacement at this time.

Comment:
One commenter stated that damaged eyes are quickly removed and excluded from the BCOP test method and that Gautheron et al. (1992) used both fresh eyes and eyes maintained at 4ºC and found no differences in results. The commenter also asked the Panel to reconsider the use of a histopathology evaluation in the BCOP test method.

ICCVAM Response:
The final ICCVAM recommendations state that a histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the BCOP test method is conducted. Such data will allow for development of decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.

Comment:
One commenter discussed the "top-down" (i.e., screening for corrosives/severe irritants) and "bottom-up" (i.e., screening for substances not labeled as irritants) approaches using the ICE and BCOP test methods. The commenter stated that ECVAM is developing a paper to recommend the use of these testing strategies for both ICE and BCOP. Substances could be tested in the BCOP or ICE test methods to identify corrosives/severe irritants or substances not labeled as irritants without using an animal test.

ICCVAM Response:
ICCVAM previously recommended the ICE and BCOP test methods for use in a tiered-testing strategy, where positive substances can be classified as ocular corrosives/severe irritants without the need for animal testing (ICCVAM 2006b). Based on the current evaluation of available data and corresponding performance, the original ICCVAM recommendations for the use of the BCOP and ICE test methods to identify substances as ocular corrosives/severe irritants remains unchanged.

Comment:
One commenter questioned the need for performance standards for the CM test method, given that the Panel did not recommend performance standards for the BCOP and ICE test methods.

ICCVAM Response:
The final ICCAM recommendations state that the development of performance standards for the CM test method is not warranted at this time.

Comment:
One commenter indicated that it was appropriate to include EO data that used a different protocol as a measure of test method reproducibility.

ICCVAM Response:
As stated in the AMCP SRD, ICCVAM notes that the reproducibility of the EO test method is based on an EO protocol that uses relative percent viability to assign an irritancy classification (irritant or nonirritant). It does not use a calculated ET_{50} value to predict multiple ocular hazard categories (i.e., EPA Categories I–IV). The latter is the protocol included in the AMCP BRD.

Comment:
One commenter noted that a small change in classification is seen when the BCOP test method decision criterion is changed from 55 to 75. ECVAM considers 55 the best cut-off for their intended purpose.

ICCVAM Response:
ICCVAM notes that using alternative decision criteria to identify ocular corrosives/severe irritants does not improve BCOP test method performance (i.e., IVIS $\geq$ 75, proposed in the AMCP BRD, instead of IVIS $\geq$ 55.1, per the ICCVAM-recommended BCOP protocol).

Comment:
One commenter responded to the concern about the limited number of AMCPs tested, stating that most industrial-strength cleaners are severe irritants and household cleaners are mostly mild irritants. Very few AMCPs are in the moderate range.

ICCVAM Response:
As outlined in the final AMCP SRD, only 28 AMCPs have been tested in both the BCOP and EO test methods. Of these, Draize rabbit eye test data classified only one as an EPA Category II substance and only four as EPA Category III substances. Therefore, ICCVAM concludes that although the performance of the alternate AMCP testing strategy using the BCOP and EO test methods appears useful for identifying EPA Category I substances using the BCOP test method and EPA Category IV substances using the EO test method, the data are not sufficient to adequately demonstrate that this strategy can identify all four EPA ocular hazard categories.

4.6 Public Comments in Response to 74 FR 19562 (April 29, 2009) Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

NICEATM announced the SACATM meeting (June 25–26, 2009) and requested written and public oral comments on the agenda topics.

NICEATM received four comments. Two written comments were received before the meeting, and two oral comments were provided at the SACATM meeting.

Public Responses (written)

Two written public comments were relevant to the AMCP testing strategy or one of the three *in vitro* test methods (i.e., BCOP, CM, and EO) included in the AMCP testing strategy.

Comment:

One commenter strongly supported the EPA's implementation of a pilot program for ocular safety labeling for AMCPs. The commenter suggested reserving ICCVAM reviews for tests/strategies with multi-agency applicability and adopting a streamlined approach to agency acceptance of methods/strategies deemed scientifically valid in other regions of the world.

ICCVAM Response:

ICCVAM encourages industry to generate more data using alternative *in vitro* test methods. Thus, EPA's pilot program for ocular safety labeling for AMCPs, which encourages industry to generate and submit data using the test methods in the AMCP testing strategy, should produce important data for use in future evaluations.

Comment:

One commenter commented on (1) the reason for the extensive peer review of the AMCP submission and lack of communication with the consortium regarding this evaluation, (2) the review of the validation status of the LVET, and (3) the need to change the scoring system of the LVET to replicate the Draize rabbit eye test results.

ICCVAM Response:

NICEATM requested additional information and communicated issues and data gaps to representatives of the consortium on several occasions before the Panel meeting. Because the LVET is not a validated *in vivo* reference test method, ICCVAM felt it necessary to evaluate the LVET for this purpose before using it as the basis for evaluating the validation status of the CM test method, where *in vitro* results for AMCPs were compared exclusively to LVET data. The Panel stated that the currently utilized Draize scoring system is not considered relevant to the LVET because it uses 10% of the volume. In this regard, the Panel highly recommended development of a more appropriate scoring/classification system for the LVET. However, the Panel recommended using existing data for a statistical analysis to develop such a classification system.

Public Responses, Oral

Two oral public comments were relevant to the AMCP testing strategy or one of the three *in vitro* test methods (i.e., BCOP, CM, and EO) included in the AMCP testing strategy.

Comment:

One commenter indicated that there was no need for the substances to be tested in all three of the *in vitro* test methods in the AMCP testing strategy. The commenter also suggested that test method developers be allowed greater interaction with the Panel.

ICCVAM Response:

Given the limitations of the available database for the three *in vitro* test methods, both ICCVAM and the Panel concluded that the data were not sufficient to support the AMCP testing strategy in terms of the proposed test method usefulness and limitations (i.e., the classification of substances in all four EPA ocular hazard categories). The agenda for the public peer review panel meeting included 10 opportunities for public comment, after which the Panel was asked if it had any questions for the commenter. As explained during the Panel orientation session before the meeting, the Panel Chair has the prerogative to invite additional discussion between the Panel and public commenters/invited experts.

Comment:
One commenter questioned the reason for the extensive peer review of the AMCP submission, including review of the validation status of the LVET and other test methods, when the EPA nominated only the AMCP testing strategy.

ICCVAM Response:
The charge to the Panel was clearly communicated, including the specific charge that the EPA and the consortium requested of NICEATM-ICCVAM. Given that convening a Panel meeting is a very expensive, time-consuming process, NICEATM-ICCVAM wanted to take advantage of this international Panel of experts to review other related test methods. It resulted in an aggressive agenda, but the Panel was very thorough and took the time for a careful, comprehensive review that has benefited the entire effort in this area.

SACATM Response
In general, SACATM was pleased overall with the Panel report. One SACATM member expressed the need for harmonization in the assessment of performance standards. Another SACATM member said the focus should be on the GHS system since it will ultimately be adopted. Another SACATM member expressed concern regarding the availability of the CM instrument.

4.7 Public Comments in Response to 74 FR 33444 (July 13, 2009) Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments

NICEATM requested submission of written public comments on the independent scientific peer review panel report.

No public comments were received.

5.0 References

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